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Genetic Differentiation of Populations of a Hydrothermal Vent-Endemic Gastropod, *Ifremeria nautilei*, between the North Fiji Basin and the Manus Basin revealed by Nucleotide Sequences of Mitochondrial DNA

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ABSTRACT—The genetic differentiation of populations of a hydrothermal vent-endemic gastropod, *Ifremeria nautilei*, between two back-arc basins in the south Western Pacific, namely the Manus Basin and the North Fiji Basin, was analyzed on the basis of nucleotide sequences of the mitochondrial gene for cytochrome oxidase I. The two populations of *I. nautilei* had no common haplotypes and appeared, therefore, to be isolated from one another. All haplotypes obtained from the North Fiji Basin formed a monophyletic group supported by a high bootstrap probability and the genetic diversity of the population in the North Fiji Basin was much smaller than that of the population in the Manus Basin. The population in the North Fiji Basin might have been founded by relatively recent migrants from the Manus Basin. The present results suggest that the larval dispersal ability of *I. nautilei* might be lower than that of an undescribed species in the closely related genus *Alviniconcha*.

INTRODUCTION

Many hydrothermal vents with associated chemoautosynthesis-based faunal communities have been discovered in the south Western Pacific back-arc basins, namely, the Mariana Trough (Craig *et al*., 1987; Hessler and Lonsdale, 1991), the Manus Basin (Both *et al*., 1986; Tufar, 1990; Auzende *et al*., 1997), the North Fiji Basin (KAIYO 87 Shipboard Party, 1988; Desbruyères *et al*., 1994) and the Lau Basin (NAUTILAU Group, 1990; Desbruyères *et al*., 1994). The chemoautosynthesis-based faunal communities are composed of many endemic groups, such as vestimentiferan tube worms, large bivalves of the genera *Calyptogena* and *Bathymodiolus*, and crabs of the genus *Bythograea* (Tunnicliffe *et al*., 1998). Within such communities, those in the south Western Pacific are unique with respect to species composition (Tunnicliffe and Fowler, 1996). In the vent areas in the south Western Pacific, the communities are dominated by large endemic gastropods (Both *et al*., 1986; Okutani and Ohta, 1988), which live in symbiosis with chemosynthetic bacteria (Stein *et al*., 1988; Endow and Ohta, 1989; Windoffer and Giere, 1997). Furthermore, bivalves of the genus *Calyptogena*, which form a dominant group in the communities in both the Eastern Pacific and the north Western Pacific, have not been found in the south Western Pacific, with the exception of an undescribed species collected in the Desmos Cauldron of the Manus Basin (Ohta *et al*., 1997) and the New Ireland Basin (Herzig *et al*., 1994).

The dominant gastropods in the vent areas in the south Western Pacific have been classified into two genera in the family Provannidae, namely *Alviniconcha* and *Ifremeria*. Although *Alviniconcha* gastropods have been reported from the Mariana Trough, the Manus Basin, the North Fiji Basin and the Lau Basin, *Ifremeria* gastropods have not been discovered in the hydrothermal vent fields in the Mariana Trough (Bouchet and Warén, 1991; Warén and Bouchet, 1993). The absence of *Ifremeria* gastropods in the Mariana Trough might be attributable to lower larval dispersal ability of *Ifremeria* than *Alviniconcha*. The morphology of the larval shell suggests that *Alviniconcha* gastropods undergo planktotrophic development (Warén and Bouchet, 1993). The high-dispersal mode of

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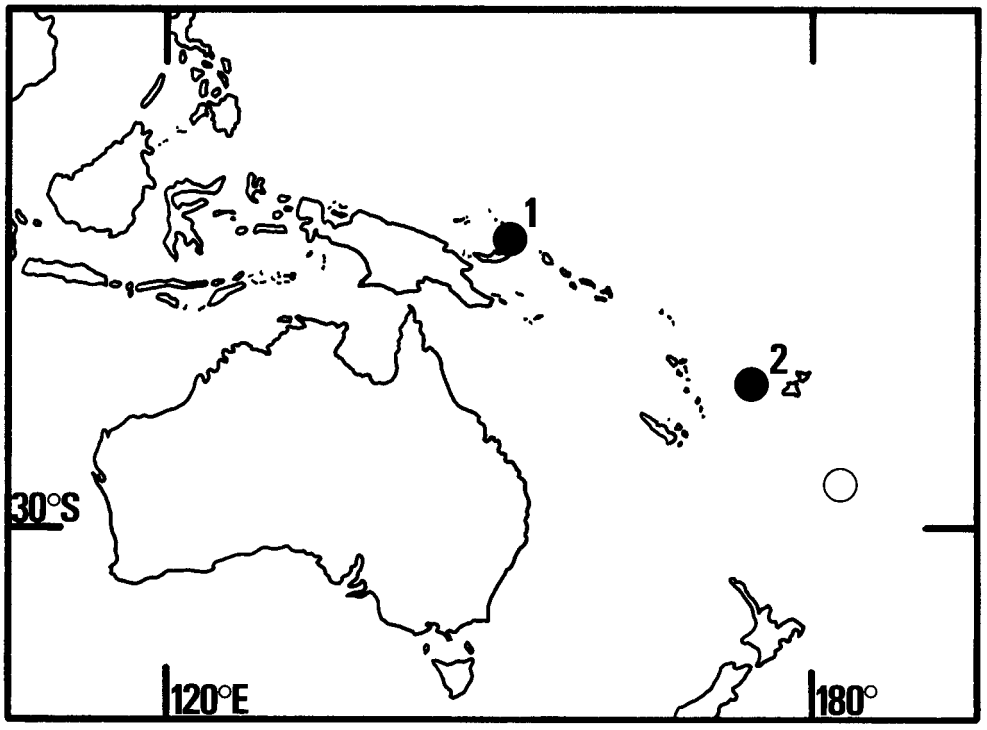

Fig. 1. Location of sites at which samples of *Ifremeria nautilei* were collected: 1, Manus Basin; 2, North Fiji Basin. An open circle denotes the location of the Lau Basin.

development is thought to have been resulted in a wide geographical distribution of *Alviniconcha* gastropods. The degree of genetic differentiation between conspecific populations is expected to be closely related to the dispersal ability of the species. As expected from the high dispersal ability of the genus *Alviniconcha*, the absence of genetic differentiation between populations in the North Fiji Basin and the Manus Basin of an undescribed species in the genus *Alviniconcha*

Table 1. List of samples.

Location	Site	Depth (m)	Submersible	Dive#	N
North Fiji Basin	White Lady site	1970	Nautile	D11	1
				D12	3
			Shinkai 6500	D77	9
				D78	1
				D80	3
	STARMER II site	1980	Nautile	D15	2
				D20	3
Manus Basin	PACMANUS site	1630	Shinkai 2000	D911	4
				D914	3
				D922	8
				D1062	9
				D1063	3
				D1067	3

Table 2. Primers used in the present study. Y, R, S and W denote T or C, A or G, G or C, and A or T, respectively. Positions refer to the corresponding amino acid residues encoded by the gene for mitochondrial cytochrome oxidase I from *Drosophila yakuba*.

Name	Sequence	Position	Direction
COI-B	5'-GGATGAACNGTNTAYCCNCC-3'	123–129	Forward
Gastro-3	5'-TTAGCTGGTGCTTCNTCNATYYTNGG-3'	150–158	Forward
COI-3	5'-GTNTGRGCNCAYCAYATRTTYACNGT-3'	285–293	Forward
TW-2	5'-ACTACRTARTANGTRTCRTG-3'	366–372	Reverse
COI-6	5'-GGRTARTCNSWRTANCGNCGNGGYAT-3'	434–442	Reverse

was reported (Kojima *et al.*, 1998).

The larval type of *Ifremeria* gastropods remains unknown because of corrosion of the protoconch of all available specimens (Warén and Bouchet, 1993). In addition, no analysis of the genetic differentiation between populations of *Ifremeria* gastropods has been reported. In the present study, we analyzed the genetic differentiation of populations of *I. nautilei* between the Manus Basin and the North Fiji Basin (Fig. 1) by examining nucleotide sequences of mitochondrial DNA in an effort to characterize the larval dispersal ability of *Ifremeria*.

MATERIALS AND METHODS

During dives of the submersible "Nautile" of Institut français de recherche pour l'exploitation de la mer (IFREMER) and of the submersibles "Shinkai 2000" and "Shinkai 6500" of the Japan Marine Science and Technology Center (JAMSTEC), a total of 52 specimens of *Ifremeria nautilei* was collected, as summarized in Table 1. One specimen of *Alviniconcha hessleri*, collected at the Forecast Vent site on the South Mariana Ridge (1470 m depth) during a dive of "Shinkai 6500" (No. D186), was used as the outgroup for phylogenetic analysis. Warén and Bouchet (1993) examined young specimens and the anatomy of *A. hessleri* and *I. nautilei*, and they stated that the genera *Alviniconcha* and *Ifremeria* are closely related and that these genera should be grouped into the family Provannidae. Beck (1991) also stated that *Alviniconcha* and *Olgaconcha* (=*Ifremeria*) are closely related to one another.

Mitochondrial DNA (mtDNA) was extracted from the deep-frozen head-foot region of each individual by a modified version of the method described by Komm *et al.* (1982). In the case of some damaged samples, which had been collected in the North Fiji Basin in 1990 and stored at –20°C, total DNA was extracted by grinding of

Fig. 2. Nucleotide sequences of mitochondrial genes for cytochrome oxidase I from *Ifremeria nautilei* (I) and *Alviniconcha hessleri* (A). Asterisks indicate nucleotides that are identical to those in I. Numbers above the sequence from *I. nautilei* denote positions at which intraspecific variations were observed. Y, R, S and W denote T or C, A or G, G or C, and A or T, respectively. Underlining indicates sites at which amino acid substitutions were identified. Positions of primers COI-3 and TW-2 are shown. The nucleotide sequences will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases under accession numbers AB010097 (I) and AB010094 (A).

samples, digestion with sodium dodecyl sulfate (SDS) and proteinase K, and extraction with phenol and chloroform. In the case of samples collected in the Manus Basin in 1998, total DNA was isolated from samples without digestion with proteinase K.

A fragment (about 450 bp) of the mitochondrial gene for cytochrome oxidase I (COI) was amplified by the polymerase chain reaction (PCR) with "universal" primers for amplification of most genes for metazoan COI, COI-3 and COI-6 (Shimayama *et al*., 1990). The conditions for PCR were as follows: 94°C for 60 s; then 30 to 40 cycles at 92°C for 40 s, 40°C for 60 s, and 72°C for 90 s. Genereleaser™ (BioVenture Inc., Murfreesboro, TN, USA) was used to sequester products of cell lysis that might have inhibited the polymerase. The nucleotide sequences of fragments (306 bp) were determined by the dideoxynucleotide chain-termination method using a Sequenase™

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                                     Site
              1111111111222222222233333333334444444444555555555
Type 1234567890123456789012345678901234567890123456789012345678     N
M1   CAGACATGGTTGTGTGTCCTTCTGTGAAGCATGGCTTCGACACGGCTGTCTCGTTACG     5
M2   ***G******************************************************     1
M3   ******************T***************************************     2
M4   ********************************A*************************     1
M5   **A*******************************************************A     1
M6   ********************C*C***********************************     1
M7   *******A*****************************T**************A*****     1
M8   *G*G*************T****************T***********************     1
M9   ****TT************************G****C****************A*****     1
M10  ****T********************A****G****C****************A*****     1
M11  ****T*************************G****C*************T**A*****     1
M12  T***T*************************G****C****T***********A*****     1
M13  ****TT************************G****C******T*********A*****     1
M14  ****T******A******************G****C***************TA*****     1
M15  ****T**********A************A*G****C****************A*****     1
M16  ****T**********A**************G****C****************A**G**     1
M17  ****T************T*****************C*******A*****T**A*****     1
M18  ****T******************A*A****G****C****************A*****     1
M19  ***********A******************G******T*****A*****T**A*****     1
M20  T***T*************************G****C****TG**********A*****     1
M21  ****TT**A****A****************G****C****************A*****     1
M22  T***T************T********G***G****C***************TA*****     1
M23  T***T*C**********T********G***G****C***************TA*****     2
M24  ****T**************C****CA****G****C****T*****C*****A*****     1
F1   ************C*C******G********G********G*******A**********     1
F2   ************C*C******G********G********G*******A*****C****     1
F3   **********C*C*C******G********G********GT******A**********     1
F4   ************C*C******G*****G**G********G*******A****A*****     4
F5   ************C*C******G*******TG********G*******A****A*****     1
F6   ****T*******C*C******G*****G**G********G*******A****A*****     1
F7   ************C*C******G*****GA*G********G*******A****A*****     1
F8   ************C*C******G*****G**G*****C**G*******A****A*****     1
F9   ************C*C******G*****G**G*******AG*******A****A*****     1
F10  ************C*C******G*****G**G********G****A**A****A*****     1
F11  ************C*C******G*****G**G********G*****T*A****A*****     2
F12  ************C*C******G*****G**G********G*******AC***A*****     1
F13  ************C*C******G*****G**G********G*******A****A*C***     1
F14  ************C*C******G*****G**G********G*******A****A***T*     1
F15  T***********C*C******G*****G**G*******AG*******A****A*****     1
F16  *********C**C*C******G*****G**G*******AG*******A****A*****     1
F17  ************C*C******G*****G**GC*******G*******A**C*A*****     1
F18  ****T*******C*C*C****G*****G**G**A*****G*******A****A*****     1
```

Fig. 3. Nucleotides at variable positions in the sequence of the gene for COI from *Ifremeria nautilei*. Numbers refer to the positions shown in Fig. 2. Asterisks indicate nucleotides that are identical to those in the uppermost sequences. M and F, as prefixes in names of haplotypes, denote haplotypes obtained from specimens from the Manus Basin and those from the North Fiji Basin, respectively.

PCR product sequencing kit (United States Biochemical Coop., Cleveland, OH, USA). A longer fragment (about 960 bp) of genes for COI was amplified by PCR using primers COI-B (Hasegawa *et al*., 1996) and COI-6. The conditions for PCR were as follows: 94°C for 60 s; then 30 to 40 cycles at 92°C for 40 s, 50°C for 60 s, and 72°C for 90 s. Nucleotide sequences within the upper region of this fragment were determined with a sequencer (DSQ-2000L; Shimazu Corp., Kyoto, Japan) using primers TW-2 (Kojima *et al*., 1997) and Gastro-3. The sequence of primer Gastro-3 was based on sequences from some specimens of *I. nautilei* that had been determined using primer COI-B (data not shown). Nucleotide sequences of primers used in the present study are summarized in Table 2. Amino acid sequences of COI were deduced by reference to the modified genetic code of molluscan mtDNA (Shimayama *et al*., 1990; Hoffmann *et al*., 1992).

The genetic distance between sequences was calculated by Kimura's two-parameter method (Kimura, 1980). Phylogenetic trees were constructed by the neighbour-joining method (Saitoh and Nei, 1987) with the program from the MEGA package, Version 1.0 (Kumar *et al*., 1993) and by the maximum parsimony method with the computer program Parsimony, which was provided by Dr. K. Tamura (Tokyo Metropolitan University). Number of net nucleotide substitution between populations (Nei, 1987) was estimated on the basis of numbers of nucleotide substitution between haplotypes calculated by Kimura's two-parameter method (Kimura, 1980). Genetic diversity of populations was estimated in terms of two indices: gene diversity (Nei, 1987) and nucleotide diversity (Tajima 1983; Nei, 1987). Estimations were made with the computer program Arlequin (Schneider *et al*., 1996). Gene diversity is the probability that two randomly chosen haplotypes are different. Nucleotide diversity is the probability that two randomly chosen homologous nucleotides are different.

RESULTS

Partial sequences (792 bp) of mitochondrial genes for COI were determined for 52 specimens of *Ifremeria nautilei* and one specimen of *Alviniconcha hessleri* (Figs. 2 and 3). Two intraspecific amino acid substitutions were detected in the sequences from the specimens of *I. nautilei*. Each amino acid substitution was detected in the case of only one specimen collected in the Manus Basin. Six amino acid substitutions were detected when we compared the dominant sequence from *I. nautilei* and the sequence from *A. hessleri* (Fig. 2).

Within the sequences of *I. nautilei*, 58 sites were found to

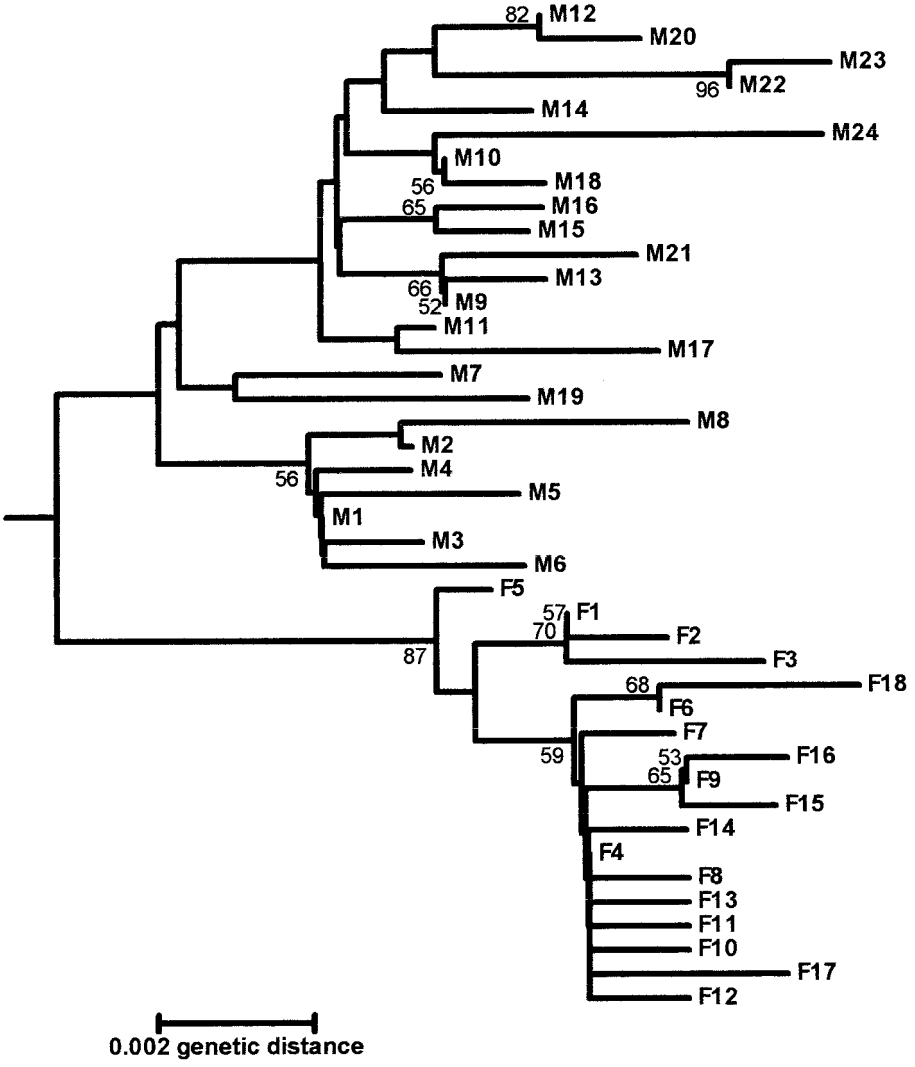

Fig. 4. Phylogenetic tree for haplotypes of *Ifremeria nautilei*. The tree was constructed by the neighbour-joining method with *Alviniconcha hessleri* as the outgroup. Bootstrap values are shown below branches of clades that are supported by bootstrap values of more than 50%.

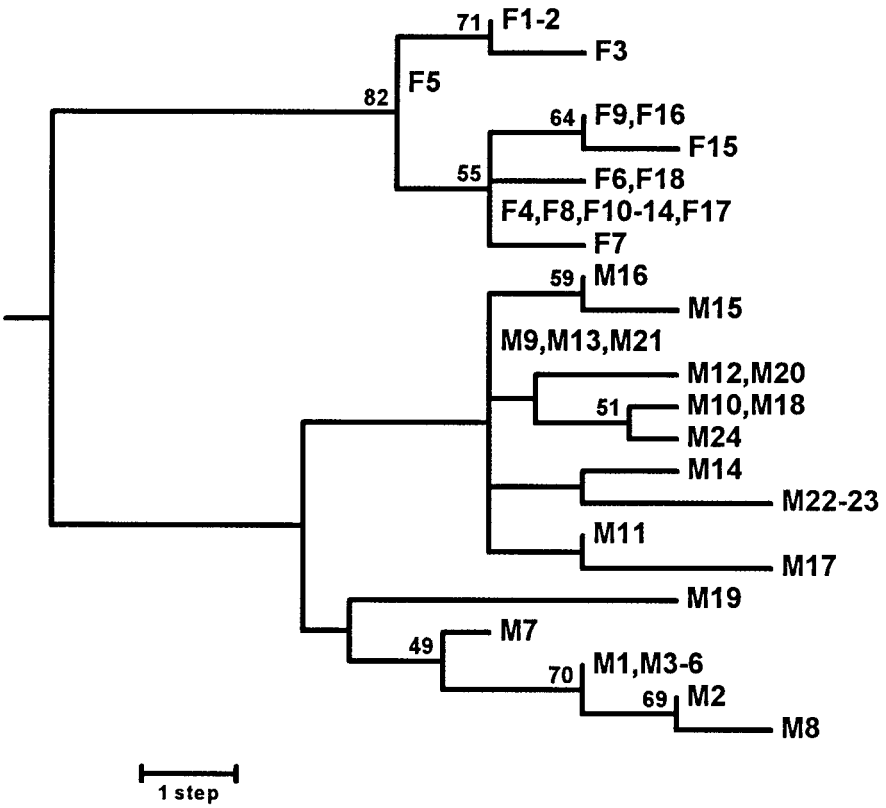

Fig. 5. A 50% majority rule consensus phylogenetic tree for haplotypes of *Ifremeria nautilei*. The tree was constructed by the maximum parsimony method with *Alviniconcha hessleri* as the outgroup. Bootstrap values are shown above branches of clades that are supported by bootstrap values of more than 50%.

Table 3. Genetic diversity of populations of *Ifremeria nautilei*, as estimated from two indices, with standard deviations.

Diversity index	Population	
	Manus Basin	North Fiji Basin
Gene diversity	0.97±0.02	0.97±0.03
Nucleotide diversity	0.0075±0.0041	0.0032±0.0020

be polymorphic and 42 haplotypes were obtained from 52 individuals (Fig. 3). Between the population of *I. nautilei* in the North Fiji Basin and that in the Manus Basin, five nucleotide substitutions were fixed (Fig. 3) and the two populations had no common haplotypes.

The phylogenetic relationships among the deduced haplotypes of *I. nautilei* were analyzed by the neighbour-joining (NJ) method and the maximum parsimony (MP) method. Individuals collected in both basins formed reciprocally monophyletic clusters on the NJ tree (Fig. 4). While monophyly of haplotypes from the North Fiji Basin was supported by a high bootstrap probability (88%), that of haplotypes from the Manus Basin was supported by a very low bootstrap probability (26%). On the MP tree (Fig. 5), haplotypes from the North Fiji Basin formed a monophyletic cluster supported by a high bootstrap probability (82%) and a bootstrap probability supporting the monophyly of haplotypes from the Manus Basin was low (45%).

The number of net nucleotide substitution between two populations was estimated to be 0.0082. The genetic diversity of the two populations is summarized in Table 3. While gene diversity values were almost the same in the Manus Basin and the North Fiji Basin, the genetic diversity in the Manus Basin, as estimated in terms of nucleotide diversity, was about twice that in the North Fiji Basin. These results mean that diversity of haplotypes was the same in both populations but the average extent of genetic divergence among individuals was much greater in the Manus Basin than in the North Fiji Basin.

DISCUSSION

In the present study, we analyzed the genetic differentiation between the Manus Basin and the North Fiji Basin of populations of *Ifremeria nautilei*, one of the dominant species in the hydrothermal areas in the south Western Pacific. We found that these populations had no common haplotypes and that five nucleotide substitutions were fixed between them (Figs. 2 and 3). Thus, they appeared to be geographically isolated from one another.

For 20 and 12 individuals of an undescribed species in the genus *Alviniconcha* in the Manus Basin and the North Fiji Basin, respectively, Kojima *et al.* (1998) determined nucleotide sequences of the same region of the mitochondrial gene for COI as the region analyzed for *I. nautilei* in the present study. Both the exact test of population differentiation

(Raymond and Rousset, 1995) and the randomized chi-squared test of independence (Roff and Bentzen, 1989) showed the absence of genetic differentiation between populations of *Alviniconcha* sp. in the Manus Basin and the North Fiji Basin (Kojima *et al*., 1998).

The undescribed species in the genus *Alviniconcha* and *I. nautilei* live close to the same hydrothermal vents in the both basins. Thus, the present results suggest that the larval dispersal ability of *I. nautilei* might be lower than that of *Alviniconcha* sp. While *Alviniconcha* gastropods were shown to undergo planktotrophic development, the larval type of *Ifremeria* gastropods remains unknown (Warén and Bouchet, 1993). To determine the larval type of *Ifremeria* gastropods, it is necessary to collect larvae or juveniles at early development stages for direct observations using a submersible with a sampling system such as a slurpgun. The analysis of DNA sequences should be very useful for identification of such samples (Dixon *et al*., 1995).

On the neighbour-joining tree (Fig. 4) and the maximum parsimony tree (Fig. 5), all haplotypes of specimens of *I. nautilei* obtained from the North Fiji Basin formed a monophyletic cluster that was supported by high bootstrap probabilities. The cluster from the Manus Basin was supported only by very low bootstrap probabilities and, therefore, the monophyly of individuals from the Manus Basin is open to question. They might be paraphyletic with exclusion of individuals from the North Fiji Basin.

The genetic diversity of the population of *I. nautilei* in the North Fiji Basin was much smaller than that of the population in the Manus Basin. The limited genetic divergence and the clear monophyly of haplotypes from the North Fiji Basin can be explained if we postulate that *I. nautilei* in the North Fiji Basin is derived from a relatively recent dispersion from another basin. If the population in the North Fiji Basin was founded by a few immigrants and if the time since their arrival has been insufficient for creation of high genetic diversity via the introduction of genetic mutations, the genetic diversity of this population would be expected to remain at a low level. Since the environments around hydrothermal vents are unstable, it is also likely that large fluctuations in population size have occurred very frequently. If the population of *I. nautilei* in the North Fiji Basin were to have recently experienced a severe decrease, such a decrease might also have reduced the genetic diversity of the population. As *I. nautilei* and *Alviniconcha* sp. inhabit the same vent areas in the both basins, the genetic diversity of the population of *Alviniconcha* sp. in the North Fiji Basin should be lower than that in the Manus Basin, if low genetic diversity of the population of *I. nautilei* in the North Fiji Basin is attributed to the bottleneck effect caused by the change of the environments in the North Fiji Basin. Between the population of *Alviniconcha* sp. in the Manus Basin and that in the North Fiji Basin, little difference in the genetic diversity was shown in terms of both gene diversity (0.86±0.06 for the Manus Basin and 0.85±0.10 for the North Fiji Basin) and nucleotide diversity (0.0035±0.0022 for the Manus Basin and 0.0030±0.0020 for the North Fiji Basin) (unpublished data). The decrease of population size in the past seems unlikely to explain the low genetic diversity of the population of *I. nautilei* in the North Fiji Basin.

The hydrothermal areas in the Manus Basin is the westernmost habitat of *I. nautilei* and the nearest habitat is situated in the North Fiji Basin. The St. Georges Undercurrent and the Equatorial Undercurrent flow from the Manus Basin to the North Fiji Basin (Butt and Lindstrom, 1994). If individuals of *I. nautilei* in the Manus Basin are paraphyletic, with exclusion of individuals in the North Fiji Basin, it seems likely that the population in the North Fiji Basin was founded by immigrants from the Manus Basin and that the two populations have not yet become reciprocally monophyletic, even though each population has been accumulating genetic differences. If the rate of sequence divergence of the COI gene of snapping shrimps, namely 0.014 per million years (Knowlton and Weigt, 1998) is used, we can estimate that the divergence between the population in the North Fiji Basin and that in the Manus Basin occurred 0.59 million years ago. Such a time frame suggests that the recruitment of *I. nautilei* to the North Fiji Basin was a relatively recent event.

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